

***Erysiphe bistortae* sp. nov. on *Bistorta amplexicaulis* from Pakistan**

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ABSTRACT—A powdery mildew fungus appearing on the leaves of *Bistorta amplexicaulis* in Pakistan is described as a new species, *Erysiphe bistortae*. This species is characterized by its large-sized chasmothecia and relatively small conidiophores. Because of biological and morphological differences from related species, supplemented by results of molecular sequence analysis, the Asian powdery mildew on *B. amplexicaulis* warrants description as a species of its own.

KEY WORDS—*Erysiphaceae*, *Polygonaceae*, taxonomy

Introduction

Polygonaceae is a cosmopolitan family comprising roughly 12,000 species and 48 genera (Sanchez & Kron 2008). They are an assemblage of morphologically diverse trees, shrubs and herbs (Hutchinson & Dalziel 1954; Brummitt 1992). Their distribution is worldwide from the tropics to the arctic, yet a large proportion of the species are concentrated in the northern temperate region (Heywood 1978). This family is represented in Pakistan by 19 genera and 103 species, with the genus *Bistorta* (L.) Scop. represented by seven species (Qaisar 2001), including *B. amplexicaulis* (D. Don) Greene. *Bistorta amplexicaulis* is a perennial shrub, with a Pakistani distribution in Chitral, Swat, Kashmir, and Murree Hills (Nasir & al. 1972). Its leaves are used for making tea which is exceptionally effective for curing fever, flu, and aching joints (Qureshi & al. 2007). During a survey of powdery mildews in Azad Jammu & Kashmir and

Khyber Pakhtunkhwa, Pakistan, this economically important plant was found to be infected with a species of the genus *Erysiphe*.

Previously, only one powdery mildew species, *Erysiphe polygoni* DC., has been reported to occur on *Bistorta amplexicaulis* in Pakistan (Ahmad 1978). After detailed analysis, our newly collected specimens were found to be different from previously reported species of *Erysiphe*. This species is described morphologically along with molecular analysis, which support its identification and proposal as a new species, *Erysiphe bistortae*.

Materials & methods

Collection & Preservation

Leaves infected with powdery mildew were collected during the phytopathological survey of Azad Jammu & Kashmir and Khyber Pakhtunkhwa, Pakistan, in 2020. The study areas lie in the north-eastern part of Pakistan that is characterized by green, rough and undulating terrain and is floristically very rich (Dar 2012). For preservation, the infected plants were shade dried with blotting paper and further protected in envelopes. The specimens are deposited at the Herbarium of Institute of Botany, University of the Punjab, Lahore, Pakistan (LAH).

Morphological characterization

Infected samples were examined macromorphologically under a stereomicroscope (Meiji Techno, EMZ-5TR, Japan) and slides were prepared in lactic acid. The micromorphology of hyphae on the host, hyphal appressoria, conidia, conidiophores, shapes and sizes of chasmothecia, asci, and ascospores were examined under a compound microscope (SWIFT M4000-D) with a 9MP camera system. A minimum of twenty measurements were taken for each diagnostic feature.

DNA extraction, PCR amplification, phylogenetic analysis

Dried powdery mildew colonies were scratched off with the help of razor blades from fresh fungal specimens, ground in liquid nitrogen and stored in Eppendorf tubes at -18°C . DNA was extracted using Thermo Scientific GeneJET Plant Genomic DNA Purification Mini Kit #K0791. The Internal Transcribed Spacer (ITS) region was amplified using PMITS1/PMITS2 primers (Cunnington & al. 2003). PCR products were visualized on 1% agarose gel with ethidium bromide through Gel documentation system (Sambrook & Russel 2001). PCR products were sent for sequencing to Tsingke, China. By using BioEdit, raw sequenced data were edited (Hall 1999). The ITS sequences were BLAST searched against the GenBank database (www.ncbi.nlm.nih.gov). Maximum percent identity and query coverage of sequences with related taxa were noted. All sequences along with the new sequences were aligned through MAFFT (Multiple sequence alignment tool). Sequences were aligned and trimmed at conserved sites from both 5' / 3' ends. The phylogenetic tree was executed within MEGA 6.0 (Tamura & al. 2013), using Maximum Likelihood Method based on

TABLE 1. Strains and sequences used in the phylogenetic analyses of *Erysiphe* species.
New sequences are in bold.

SPECIES	COUNTRY	VOUCHER	GENBANK (ITS)
<i>E. alphitoides</i>	United Kingdom	OE2015PMCS301	KY660893
	United Kingdom	RHS287283	KP686268
<i>E. berberidicola</i>	South Korea	KUS-F30268	MG938640
	South Korea	KUS-F30028	MG938639
<i>E. betae</i>	Greece	Se1	KY399969
	China	ZKEB001A	KF268348
	Korea	KUS: F29140	KX574674
	Pakistan	AA#4 (PUP Bot.01	MN368297
<i>E. bistortae</i>	Pakistan	LAH36943	MZ048848
	Pakistan	LAH36143	MZ048849
<i>E. castaneigena</i>	Korea	KUS:F28761	KY926846
	Korea	KUS:F28676	KY926844
	Korea	KUS:F28682	KY926845
<i>E. cruciferarum</i>	United Kingdom	OE2015PMCS236	KY660884
	United Kingdom	OE2015PMCS250	KY660924
<i>E. diffusa</i>	Spain	Erd1	MK673961
<i>E. euonymicola</i>	United Kingdom	OE2016PMCS1	KY661150
<i>E. heraclei</i>	Argentina	MUMH<JPN>:1874	LC010004
	Korea	KUS-F27279	KF111807
	China	HMNWAFU-CF2010265	KR048065
	Australia	BRIP 68828	MT174194
	Australia	BRIP 59438	MT174193
	Korea	KUS:F28393	KP729443
	United Kingdom	OE2015PMCS211	KY660878
	United Kingdom	OE2016PMCS5	KY661135
	United Kingdom	OE2014PM79CS	KY660797
<i>E. intermedia</i>	United Kingdom	OE2015PMCS297	KY660904
	USA	490P06380636	MK094072

SPECIES	COUNTRY	VOUCHER	GENBANK (ITS)
<i>E. ludens</i>	United Kingdom	OE2015PMCS298	KY660905
	United Kingdom	OE2015PMCS259	KY660901
<i>E. malvae</i>	Ukraine	MUMH<JPN>:2570	LC010041
	Ukraine	MUMH<JPN>:2569	LC010040
<i>E. mori</i>	China	Ma001	KY910120
<i>E. pisi</i>	United Kingdom	OE2013PM18	KY660750
	United Kingdom	OE2016PMCS80	KY653209
<i>E. polygoni</i>	Azerbaijan	MUMH<JPN>:7036	LC328322
	Azerbaijan	MUMH<JPN>:7045	LC328323
	China	PM	MW169023
	—	—	AF011307
	United Kingdom	OE2016PMCS63	KY661154
<i>E. trifoliorum</i>	United Kingdom	OE2015PMCS231	KY660892
	United Kingdom	OE2015PMCS265	KY660909
<i>E. tortilis</i>	United Kingdom	OE2014PM83CS	KY660755

Kimura 2-parameter with 1000 rapid bootstrap replicates. The model of evolution was selected by searching for the best DNA model for ML analysis in MEGA 6.0 (Tamura & al. 2013).

Results

Taxonomy

Erysiphe bistortae Afshan, I. Zafar & Khalid, **sp. nov.**
MB839866

FIGS 1, 2

Erysiphe bistortae differs from *Erysiphe polygoni* by its larger chasmothecia; and from *E. betae*, *E. heraclei*, and *E. malvae* by its conidiophores having relatively short foot cells followed by 1–2 shorter cells.

TYPE—Pakistan, Azad Jammu & Kashmir, Las Dana, 33.92°N 73.96°E, 2500 m alt., on leaves of *Bistorta amplexicaulis* (D. Don) Greene (*Polygonaceae*), 26 Sep. 2020, N.S. Afshan and I. Zafar LDH-01 (**Holotype**, LAH36943; GenBank MZ048848).

ETYMOLOGY—the species epithet refers to the host genus, *Bistorta*.

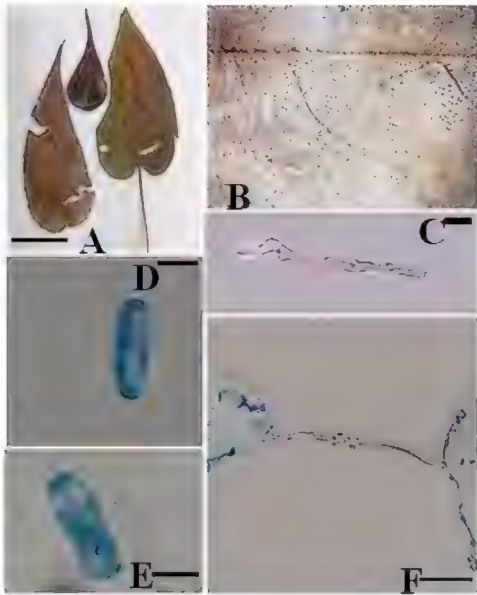


FIG. 1. *Erysiphe bistortae* (holotype, LAH 36943). A. Infected host plant; B. Infection under stereomicroscope; C. Appresorium; D, E Conidia; F. Conidiophore & foot cell. Scale bars: A = 5 cm; C–E = 15 μ m; F = 40 μ m.

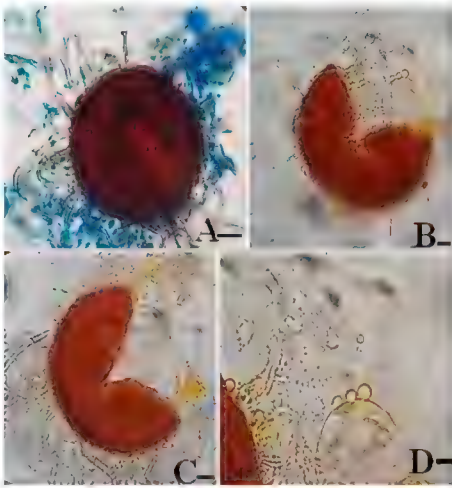


FIG. 2. *Erysiphe bistortae* (holotype, LAH 36943) A. Chasmothecium with appendages; B. Chasmothecium releasing ascospores; C. Ascus containing ascospores; D. Ascospores. Scale bars: A = 30 μ m; B, C = 20 μ m; D = 15 μ m.

MYCELIUM amphigenous, also on stems, effused or in patches, persistent, 4–7 μ m wide. HYPHAE hyaline, thin- and smooth-walled. CONIDIOPHORES erect from top of the mother cell, reaching up to a height of 107 μ m. FOOT CELLS cylindrical, straight, 13–40 $\times$ 5–10 μ m, followed by 1–2 shorter cells and single conidium. CONIDIA ovoid to cylindrical, 28–48 $\times$ 13–20 μ m, germ tubes arising from an end, short to quite long, 0.25–3.5 times the conidial width. APPRESSORIA with 0–9 lobes, variable, 3–8 μ m diam. CHASMOTHECIA scattered to gregarious, dark brown in color, globose to sub globose, 105–163(–165) μ m diam. PERIDIUM CELLS inconspicuous, irregularly hexagonal, 8–17 μ m diam. APPENDAGES numerous, variable in number, mycelioid, simple or frequently irregularly branched, often in a coral-like manner, densely crowded and interwoven mycelium, 0.5–1.4 times as long as the chasmothecial diam., 3–6(–7) μ m wide, septate, often with only a few septa, thin-walled, smooth, pigmented when mature, yellow to brown throughout or paler towards the tip. ASCI (2–)3–5, broad ellipsoid, obovoid, saccate, 44–75 $\times$ (35–)30–54 μ m, short stalked, rarely sessile, (2–)3–5-spored. ASCOSPORES ellipsoid to ovoid, 25–34 $\times$

12–17 μm , colorless.

ADDITIONAL SPECIMEN EXAMINED—PAKISTAN, KHYBER PAKHTUNKHWA, Abbottabad, Thandiani, 34.23°N 73.35°E, 2500 m alt., on *Bistorta amplexicaulis*, 13 Aug. 2020, N.S. Afshan and A.R. Niazi JP-07 (LAH36143; GenBank MZ048849).

Discussion

Two nr ITS DNA sequences of the local collections (JP-7 and LDH-01) were generated and BLAST searched at <https://blast.ncbi.nlm.nih.gov/>. These showed sequence similarity of 97.44% with *Erysiphe betae* (Vaňha) Weltzien (MN368297) and 98.40% with *E. betae* (KX574674). Closely related ingroup sequences were retrieved from GenBank and published literature (Takamatsu & al. 2015) along with an outgroup sequence *Erysiphe mori* (I. Miyake) U. Braun & S. Takam. (KY910120). All retrieved sequences, together with our new Pakistani sequences, were aligned through MAFFT (Multiple sequence alignment tool). The final data set contained 596 positions out of which 453 were conserved, 208 were variable and parsimony uninformative, and 51 were parsimony informative. The final data set was analyzed on MEGA 6.0 for assessing its correct position. The newly generated Pakistani sequences (JP-7 and LDH-01) clustered within the *E. betae*/*E. heraclei*/*E. malvae* complex lineage with a strong bootstrap support (FIG. 3). This complex consists of *E. betae* (KY399969, KX574674, KF268348, MN368297), *E. heraclei* DC. (KY661135, KY660878, LC010004, KF111807, KR048065, MT174194, MT174193, KP729443), and *E. malvae* V.P. Heluta (LC010041, LC010040). Other sequences closely related to the Pakistani collections on *Bistorta amplexicaulis* pertain to *E. polygona* (LC328322, LC328323, MW169023, AF011307, KY661154) (FIG. 3).

The new Pakistani sequences showed 98.40 and 97.7% sequence similarity with *E. betae* (KX574674, KY399969, MN368297, KF268348), 98.90% with *E. heraclei* (KY661135, KY660878, LC010004, KF111807, KR048065, MT174194, MT174193, KP729443), and 98.81% with *E. malvae* (LC010041, LC010040) within the complex; and 97.92% sequence similarity with *E. polygona* (LC328322, LC328323, MW169023, AF011307, KY661154).

The new Pakistani sequences showed two nucleotide differences from *E. heraclei* (KY661135, KY660878, LC010004), three from *E. heraclei* (KF111807, KR048065), four from *E. heraclei* (MT174194, MT174193), and five from *E. heraclei* (KP729443); two nucleotide differences from *E. betae* (KY399969, KX574674, KF268348), zero difference from *E. betae* (MN368297); two nucleotide differences from *E. malvae* (LC010041, LC010040); and nine nucleotide differences compared from *E. polygona* (AF011307), eight from

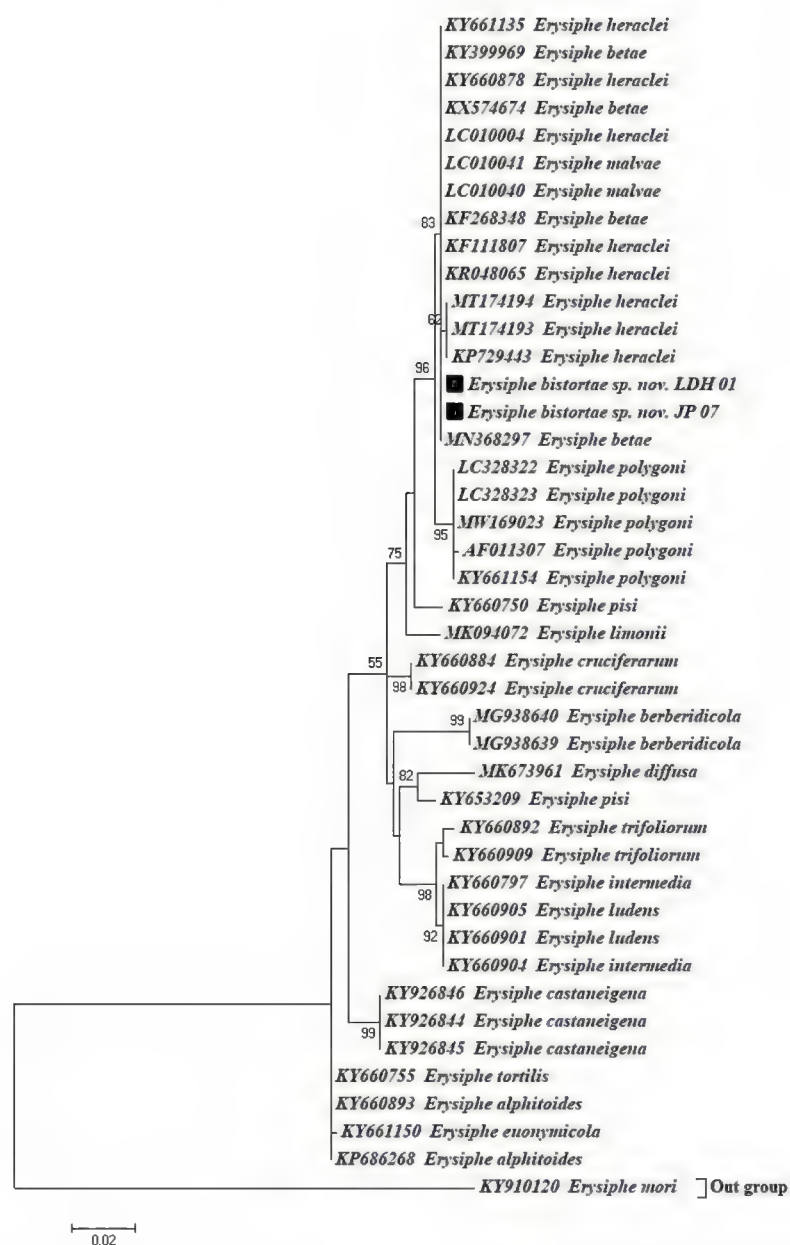


FIG. 3. Phylogenetic analysis of *Erysiphe bistortae* based on a Maximum likelihood analysis of the ITS region. The tree was rooted using *Erysiphe mori*. The ML bootstrap value is shown adjacent to each branch. The new sequence from Pakistan is marked with ■.

E. polygoni (KY661154) and seven from *E. polygoni* (LC328322, LC328323, MW169023).

Erysiphe bistortae, the new powdery mildew species on *Bistorta amplexicaulis* in Pakistan, belongs morphologically as well as genetically to the *Erysiphe betae*/*E. heraclei*/*E. malvae* complex (cluster), with *E. polygoni* as sister group (Takamatsu & al. 2015). All species included in this complex are phylogenetically closely allied and morphologically characterized by having rather similar asexual morphs and chasmothecia with frequently irregularly branched appendages. At first glance, one would be inclined to assign the

Erysiphe on *Bistorta* (*Polygonaceae*) to *Erysiphe polygoni*, described from Europe on *Polygonum aviculare* L. as type host, a species pertaining to *Polygonum* s.str. *Bistorta* spp. are unknown as hosts of this species in Europe and in general (Braun & Cook 2012). The asexual morph of *E. polygoni* agrees well with collections on *Bistorta amplexicaulis*, and the *E. polygoni* chasmothecia are also close but smaller (85–140 µm diam.; Braun & Cook 2012). *Erysiphe polygoni* is biologically specialized, and the powdery mildew on *Polygonum aviculare* s.lat., the type host, is confined to that host (= f.sp. *polygoni-avicularis*; Hammarlund 1925, Blumer 1967), and sequences retrieved from *B. amplexicaulis* cluster within the *Erysiphe betae*/*E. heraclei*/*E. malvae* complex, whereas *E. polygoni* clusters separately. The *Erysiphe betae*/*E. heraclei*/*E. malvae* complex (cluster) consists of species that cannot be clearly distinguished solely by their ITS sequences (Takamatsu & al. 2015), a situation that is comparable with the *Erysiphe aquilegiae* DC. complex (Shin & al. 2019, Bradshaw & al. 2021). However, on account of biological and morphological differences within this complex, the Asian powdery mildew on *B. amplexicaulis* warrants description as a species of its own. *Erysiphe betae* differs morphologically in having smaller chasmothecia, 75–135 µm diam., and conidiophore foot-cells predominantly formed by a short foot-cell followed by a longer second cell and 1–2 shorter cells; and *Beta* powdery mildew is biologically specialized and confined to *Beta* spp. (*Amaranthaceae*; Drandarevski 1978). *Erysiphe heraclei* is confined to hosts of the *Apiaceae*, with several formae speciales (Hammarlund 1925, Blumer 1967, Dixon 1978), and this species is morphologically distinguished from *E. bistortae* by longer conidiophore foot-cells (20–70 µm), often with a second cell longer than the foot-cell (Braun & Cook 2012). *Erysiphe malvae* differs in having longer conidiophore foot-cells, 35–70 µm, and smaller chasmothecia, 100–140 µm diam. (Braun & Cook 2012).

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